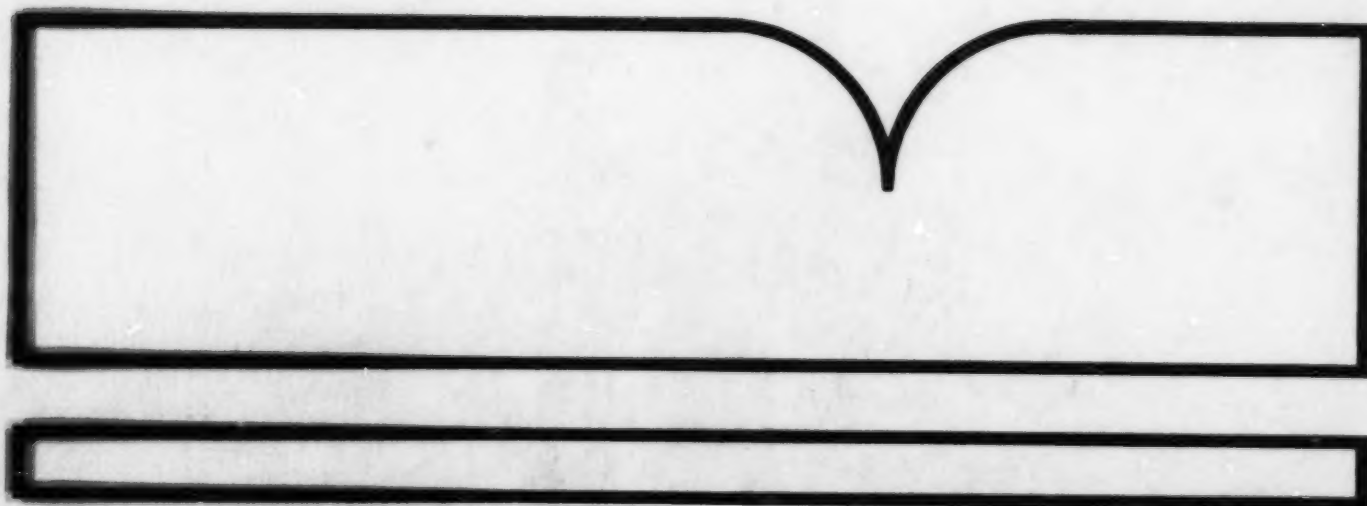


Automated Gel-Permeation System for
Removal of Lipids in Gas Chromatography/Mass
Spectrometric Analysis of Fatty Tissues for
Xenobiotic Chemicals

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16. ABSTRACT The interference of natural lipids in gas chromatography/mass spectrometric analysis of xenobiotic chemicals in fatty tissue can be substantially reduced using gel-permeation chromatographic removal of the lipids. This paper presents an inexpensive controller which can be used with any manual gel-permeation system to automate the isolation of trace chemicals from lipids. The controller is especially suited for the preparation of large samples required for gas chromatography/mass spectrometric identification of its trace contaminants.			
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Automated Gel-Permeation System for Removal of Lipids in Gas Chromatography/Mass Spectrometric Analysis of Fatty Tissues for Xenobiotic Chemicals

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Natural lipids remain one of the more difficult sources of interference in gas chromatography/mass spectrometry (GC/MS) analyses of tissue for hazardous organic contaminants. The gel-permeation chromatographic (GPC) method reported by Tindle and Stalling (1) and modified by Kuehl and Leonard (2) is widely used in GC/MS analysis of organic residues in tissues. The GPC technique in which methylene chloride is used as a solvent permits quantitative isolation of chemicals with molecular weights less than 600 from the heavier lipids regardless of the functional group of the molecule.

The primary disadvantage of the technique is the 500-mg limit to the amount of lipid that can be chromatographed as a single sample. A 100-g tissue sample containing 8% natural lipid requires at least 16 aliquots of the sample extract sequentially chromatographed over a 20-h period. Although an

automated gel-permeation system which processes up to 23 individual samples is commercially available for approximately \$9500, the unit requires that the sample be loaded into individual sample loops containing 0.5–1.0 g of lipid each. We describe herein a \$300 controller for the automation of any GPC system which sequentially injects aliquots of large lipid samples and subsequently collects the fractions containing the trace contaminants in the lipids.

EXPERIMENTAL SECTION

Materials. A conventional GPC system for preparative scale chromatography can be automated by inserting three three-way Teflon solenoids and an electronic control module in the existing system. The following materials are needed: (a) three Teflon three-way solenoids (General Valve Corp.) 12 V dc, \$45 each; (b) three Model 328A time-delay relays with mounting socket (Automatic Timing and Controls Co., King of Prussia, PA), \$46 each;

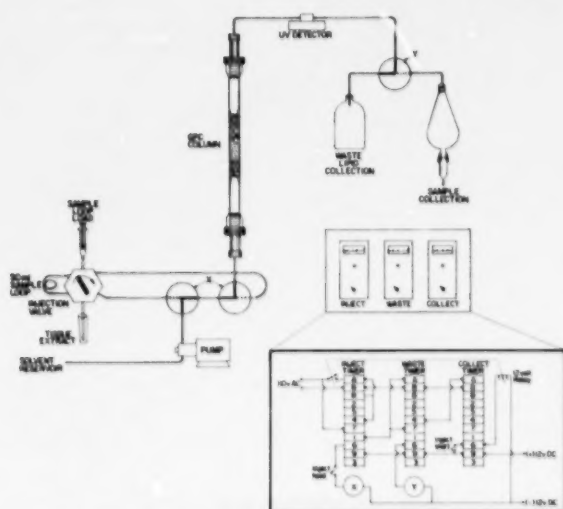


Figure 1. Electronic control module and modification of gel-permeation chromatograph.

(c) 4-A, 12-V dc power supply (Straco, Inc., Dayton, OH), Model PS-4 or equivalent, \$19.95; (d) 12-V relay, toggle switches, power cords.

Construction. A modification of the conventional GPC system used in our laboratory is shown in Figure 1. The basic system consists of a metering pump (Milton Roy), a six-position sample injector fitted with 50-mL sample loop (Cheminert), a 900 × 25 mm gel column (Ace Glass), and a UV detector (254 nm) (Varian). To automate the sample injection, we inserted two three-way solenoids in the sample loop (Figure 1). The sample is still loaded into the loop through the six-position injection valve; however, with the injector in the "inject" position, flow through the sample loop occurs only when the inject solenoids are activated. At a constant solvent flow rate, controlling the time the solenoids are activated precisely controls the volume of injection.

Automation of the fraction collection is accomplished by adding a three-way solenoid to the effluent line from the column. The normally open side of the solenoid is connected to the waste solvent receiver and the normally closed side to the sample receiver.

The electronic control module is constructed without soldering by connecting the three variable-time-delay relays in series as shown in Figure 1. The terminals (Figure 1) correspond to the

terminals on the mounting bracket of the timer. Functionally, the timers are connected as a momentary-start interval timer and two on-delay timers in series. The momentary-start timer activates the injector solenoids when the "inject start" switch is depressed. The solenoids remain activated for the duration of the timing cycle, after which power is applied to the next timer through terminal 1. The second timer is connected as an on-delay timer which activates the sample-collection solenoid at the end of the timing cycle. Thus, the adjustment of this timer controls the time between sample injection and sample collection during which the lipids elute from the column and into the waste receiver. At the end of the timing cycle the collect solenoid is activated, and power is applied to the third timer. This timer is a reset delay which allows collection of the sample until the end of the timing cycle when it activates the 12-V relay and sends a reset pulse to the first timer. When the first timer is reset, the others return to the initial conditions, and the next aliquot of the sample is injected into the column. This process cycles until the power is turned off.

Procedure. The tissue extract is concentrated to less than 50 mL in methylene chloride and centrifuged to remove particulates. After the GPC system has been flushed thoroughly and a flow rate of approximately 3.5 mL/min is attained, the injector block is moved to the load position and the 50-mL sample is drawn into the sample loop. The injector block is moved to the "inject" position, which transfers control to the inject solenoids. Initially the three timers may be set at 1.2 min, 36 min, and 30 min, respectively, which corresponds to a 1.2-min injection (4.2 mL), a 36-min waste cycle (126 mL), and a 30-min collection cycle (105 mL). After the first injection, the waste timer is adjusted so that sample collection coincides with the end of the elution of the lipids.

RESULTS AND CONCLUSIONS

The cleanup of large tissue samples for GC/MS analyses frequently requires approximately 1 week of technician time/sample when manual GPC systems are used. Automating this procedure allows each sample to be cleaned up unattended within 24 h. Moreover, if the GPC columns are adequately matched with respect to performance, a single control model will control several GPC systems simultaneously. We have used this system for the past 2 years without a single failure because of the solenoids or timing network.

LITERATURE CITED

- (1) Tindle, R. C.; Stalling, D. L. *Anal. Chem.* **1972**, *44*, 1768-1773.
- (2) Kuehl, D. W.; Leonard, E. N. *Anal. Chem.* **1973**, *50*, 182-185.

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